Effect of drought on photosynthesis and antioxidant machinery of rice variety Pusa Basmati 1718 and amelioration of drought through use of Plant Growth Promoting Rhizobacteria

Synopsis

Submitted in the Partial Fulfilment for the Requirement of

Doctor of Philosophy

in

Botany

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2025

INTRODUCTION

Plant growth and its yield is affected by different abiotic stresses like drought, flooding, heat, cold, salt, pH imbalance, nutrient imbalance and heavy metals. When there is abiotic stress, plant shows several effects. These are visible from the molecular level to morphological levels (Fahad et al., 2017). Most abiotic stresses work negatively in plants survival and reduce plants biomass and yield by up to 50%. (Thakur et al., 2010). The osmotic stress may be caused by drought, and salinity (Jaleel et al., 2009; Thakur et al., 2010). The plants try to avoid or tolerance it and it is of multigenic nature.

From the perspective of agriculture, drought is a period with below average precipitation or the result of above normal evaporation (Rollins et al., 2013). The depth of drought depends on many factors such as occurrence of rainfall, evaporative demand and moisture storing capacity of soil (Hayes et al., 2010).

Photosynthesis is one of the most important activity of plants that is affected by drought therefore, it is important to study the effect of drought on photosynthesis. Photosynthesis comprises of light and dark reactions that are both located in chloroplasts. As a result of drought, plants perceive the oxidative stress that leads to the generation of several oxidant molecules including reactive oxygen and reactive nitrogen species. Plants increase their antioxidant capacity to counter the harmful ROS and RNS. Therefore, evaluation of antioxidant response of plants is important. Drought triggers the production of ABA and causes stomata closure that leads to decreased CO_2 influx into the leaves and as a result growth of the plants is inhibited. (Mittler and Blumwald, 2015). Harsh drought also arrests photosynthesis and disrupts metabolism (Fahad et al., 2017). Lower tissue water potential occurs along with lower Rubisco activity and electron leakages from chloroplast and mitochondrial electron transport chain leads to increased ROS production and oxidative damage to cells. This stimulates the ROS scavenging enzymes (SOD, POD, CAT, APX, GR) and antioxidant chemicals e.g. carotenoids, tocopherols, Ascorbic acid, terpenoids and flavonoids. The intensity and response-rate of this antioxidative mechanism determines a plant's ability to tolerate drought.

The rhizosphere is inhabited by assorted types of microorganisms called rhizobacteria. (Mahreen et al., 2023) or plant growth-promoting rhizobacteria (PGPR).

The harmful effects of drought and heat stress on plants can be alleviated by PGPRs. PGPRs enhance

plant growth and may affect various physiological and biochemical processes including respiration, photosynthesis, translocation, ion uptake, nutrient and sugar metabolism, and phytohormones production (Farooq et al., 2009, Prasad et al., 2011; Kloepper and Scroth, 1978; Phurailatpam et al., 2022). PGPRs increase plant growth through different mechanisms such as production of growth regulators such as auxin, gibberellins and cytokinin's. (Rupaedah et al., 2014) and siderophores, phosphate solubilization, biological nitrogen fixation, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), quorum sensing (QS) signal intervention and suppression of biofilm formation, offering phytohormone, reveal antifungal activity, assembly of vaporescent organic compounds (VOCs), competition with pathogens, encourage advantageous plant-microbe collaboration etc. (Sivasakthi et al., 2014). The genera to Rhizobacteria belongs, *Alcaligenes faecalis, Bacillus licheniformis, Bacillus subtilis, Azospirillum brasilense, Bacillus megaterium, Pseudomonas, Bradyrhizobium japonicum, Xanthomonas, Rhizobium, Enterobacter cloacae, Azotobacter diazotrophicus* and*, Pseudomonas aeruginosa, Bacillus cereus* and it said to be promoted growth of plants and helps in fight against stresses (Glick and Patten, 1996; Hussain and Hasnain, 2009).

REVIEW OF LITERATURE

Drought is highly prevalent stress in India. The general mechanisms of drought responses in plants are inhibition or slowing of shoot growth, increased root architecture, membrane damage, cell wall and cuticle thickening, leaf rolling and abscission, stomatal closure etc. Molecular mechanisms involve osmolytes accumulation, protecting the conformation of proteins, buildup of secondary metabolites and defence chemicals, hormonal modulation e.g. enhancement of ABA and ethylene activity, decreased photosynthesis etc (Ferreira et al., 2020). For example, drought tolerant Heena rice variety exhibited a robust root architecture in response to drought stress as compared to drought sensitive rice variety Kiran (Tiwari et al., 2021).

In general, stress increase the activity of various antioxidant enzymes (Hasanuzzaman et al., 2013). In a study by Dalal and Tripathy (2018) in water- stressed seedlings, Chl content was decreased during greening as compared to control. Similar observation was made in many other stresses studies (Giardi et al., 1996; see review by Chaves et al., 2009). An increase in the carotenoids, flavonoids, phenolics, tocopherols, Xanthophylls and terpenoids has also been observed in several studies as a response to

stress (Kamal et al., 2023).

Plants, when subjected to stress generate oxidant molecules e.g. ROS and RON. These are highly reactive species and cause tremendous damage to the adjacent, membranes, proteins and nucleotides in the cell. To counter the oxidant molecules, plants produce antioxidant chemical and increase the antioxidant enzyme's response. In general, most of the experiments carried out in plants, an increase of antioxidant response has been observed but few studies also report the decreased activities of antioxidant enzymes depending on plant-to-plant basis. There are three enzymatic isoforms of SOD i.e. Cu/Zn-SOD, Fe-SOD and Mn-SOD (Mittler et al., 2002). In-gel assay showed that there was an increase in the activity of SOD in rice seedlings and it was mainly due to induction of one Cu/Zn-SOD isoenzyme and one Fe-SOD isoenzyme (Mittler et al., 2002). In another study, Pallavi et al., (2004) reported an increase in the concentration of superoxide anion (O_2^{-}), increased level of lipid peroxidation and a decrease in the concentration of total soluble protein and thiols in drought-stressed rice seedlings compared to controls. The concentration of H_2O_2 as well as ascorbic acid declined with imposition of drought stress, however glutathione (GSH) concentration declined only under severe drought stress. The activities of total superoxide dismutase's (SODs) as well as ascorbate peroxidase (APX) showed consistent increases with increasing levels of drought stress, however catalase activity declined.

Varity specific and developmental stage specific responses of drought are seen in rice, similar to other crops/stresses. In a study of three rice varieties (Pusa Basmati (PB), IR29- Salt sensitive; Pokkali-Salt resistant) that were subjected to drought by treatment with 20% PEG6000, SOD activity was decreased in IR29 and PB whereas it remained same in Pokkali. Same results were obtained for antioxidant enzyme Catalase activity. The MDA and H_2O_2 content were increased in all varieties but were increased to more extent in IR29 and PB as compared to Pokkali (Basu et al., 2010). In another study, drought-tolerant rice variety Heena showed more tolerance to drought and oxidative stresses than drought-sensitive variety Kiran by generating more antioxidants chemicals and adjusting various physiological parameters through modulation in phytohormone signalling genes, TFs, stress-responsive genes, and genes involved in osmotic adjustment (Tiwari et al., 2021). Transcript expression profiling, using microarray, of drought-tolerant rice N22 (DT) and drought-susceptible rice

IR64 (DS) exhibited a diverse global transcriptional response under both control and drought stress conditions. Gene Ontology (GO) enrichment analysis suggested that drought tolerance of DT was found to be linked to enhanced enzymatic activity, whereas drought susceptibility of DS was governed by significant down-regulation of transcriptional regulatory protein–encoding genes (Sangram et al., 2011). SOD-Cc2 transcript (cytosolic isoform of SOD enzyme) increased in expression drought stressed in rice seedlings of PB1 variety (Dalal and Tripathy, 2018).

Transcriptomic is a technique to catalogue all species of transcripts, including mRNAs, long noncoding RNAs and fused RNAs. Transcriptomic analysis of data is performed through gene clustering, gene ontology enrichment, GSEA, KEGG pathway analysis, promoter motif analysis. Several transcriptomics studies have been performed in rice varieties at different developmental stages (Lenka et al., 2011; Huang et al., 2014; Plessis et al., 2015; Wilkins et al., 2016; Zhang et al., 2016; Yoo et al., 2017; Liang et al., 2021).

In the leaf tissues, Kamarudin et al., (2019) observed altered abundance of LEA genes (OsLEA1, OsLEA2, OsLEA3, OsLEA4, and OsLEA5) in drought-tolerant mutant lines (MR219-4 and MR219-9.) similar to the drought-tolerant check genotype after being subjected to drought stress. A change in the expression levels of the photosynthesis genes among the genotypes under drought stress was further noted. Tolerant drought variety IR77298-14-1-2-B-10 showed a greater number of DEGs for amino acid metabolism, cell growth, hormone biosynthesis, cellular transports, signalling, transcription factors and carbohydrate metabolism in response to drought stress (Moumeni et al., 2011).

Pusa Basmati 1718 is variety of rice-growing states of Punjab, Haryana, and Delhi (Singh et al., 2018b). It has a seed-to-seed maturity of 136–138 days and produces yield ranging from 5.0 to 5.3 t/ha but has produced potential yield of up to 6.04 t/ha. It is highly resistant to bacterial blight disease as compared to the recurrent parent, PB 1121, which is highly susceptible. PB 1718 has extra-long slender grains (8.10 mm) with very good kernel length after cooking (17.00 mm), intermediate amylose (22.2%) and strong aroma. In the panel tests, PB 1718 topped the ranking among the Basmati varieties and other cultures tested for two consecutive years (2014 and 2015). It is also among the top three foreign exchange earning varieties of India. Therefore, it’s important to study this variety’s

response to drought.

Since the decrease or increase in the activities of antioxidant enzymes and antioxidant chemicals depends on the plant species, variety, developmental phase, weather conditions, and the intensity of the stress, it is important to study this response in newer varieties that are of commercial interest e.g. Pusa Basmati 1718. Climate smart agriculture is the need of the hour, on which Govt is also stressing. Therefore, the proposed study will evaluate the growth and physiological response, photosynthetic, antioxidant and transcriptomics response of PB1718 rice variety, grown in pots as a result of drought stress treatment.

Several studies have used PGPRs to ameliorate drought (Gusain et al., 2015; Kakar et al., 2016; Joshi et al., 2020; Baruah et al., 2021; Omar et al., 2021; Abd El-Mageed et al., 2022), salt (Khumairah et al., 2022) and other stresses (Mahreen et al., 2023) in rice and other crops. The consortia from best working strains available from National repositories will be prepared and their effect in various combinations, shall be evaluated in the rice variety PB1718 through determination of photosynthetic, antioxidant and gene expression parameters.

IMPORTANCE OF STUDY

Future findings of this study will facilitate to understand the mechanism of drought stress in plants and its amelioration by PGPR. This upon successful demonstration may facilitate the crop cultivation under drought conditions which are prevalent in many parts of the country and increasing in severity every year.

OBJECTIVES

Present study will be accomplished with the following objectives:

1. To study the physiological and growth parameters of rice plants under drought stress.
2. To evaluate the effect of drought stress on photosynthesis of rice plants.
3. To study the effect of drought stress on antioxidant response in rice plants.
4. To evaluate the role of selected PGPRs in mitigating the effect of rice plants under drought.
5. To perform transcriptomic analysis of rice plants under drought stress conditions.

METHODOLOGY

Experiment material and location

The study will be conducted for the evaluation of the effect of PGPR alone and in combination with drought stress at Dayalbagh Educational Institute, Dayalbagh Agra, Uttar Pradesh, India.

Soil testing will be done for assessing the suitability of the soil for rice cultivation. PB1718 variety of Rice will take as study material. Two weeks old seedling of rice will be subjected to drought stress by withholding the water supply. Data will be taken from the appropriate replicates of flag leaves at 7, 14, 21 days after stress treatment.

Following parameters will be analyse after treatment.

1. Growth and Physiological parameters-

a. Shoot length

b. Root length

c. Fresh weight of shoot

d. Dry weight of shoot

e. Fresh weight of root

f. Dry weight of root

g. No. of leaves per plant

h. Relative water content (RWC) (Barrs and Weattherley, 1962)

2. Photosynthesis parameters -

a. Total chlorophyll content (Lichtenthaler and Buschmann., 2001)

b. Total carotenoids content (Porra et al., 1949)

c. Photosynthesis parameters (by using Infrared Gas Analyzer) (as per manual)

d. Fluorescence parameters (Papageorgiou & Govindjee; Genty et al., 1989)

e. Stomatal conductance (by using Infrared Gas Analyzer; as per manual)

3. Antioxidant evaluation-

a. Proline content (Bates et al., 1973)

b. H_2O_2 content (Velikova et al.,2000)

c. Ascorbate content (Sadasivam et al., 1987)

d. Antioxidant enzyme activity (e.g.)

- SOD (superoxide dismutase EC1.15.1.1) (Scebba et al., 1999)

- POD (peroxidase EC1.11.1.7) (Velikova et al., 2000)
- CAT (catalase EC1.11.1.6) (Aebi et al., 1984)
- APX (ascorbate peroxidase EC1.11.1.11) (Nakano and Asada, 1987)

e. Total Phenolics content (Chun et al., 2003)

4. Procurement from a National Repository (e.g. NCL, Pune, or IMTECH, Chandigarh) based on Literature

Evaluation of mitigation of drought stress on rice plants upon treatment with combinations of PGPRs.

5. Transcriptomics

A. Real time qRT-PCR of drought stress responsive genes.

Modulation of transcript of photosynthesis light (Electron transport chain components), and dark reactions (C3 cycle), chloroplast biogenesis and antioxidant enzymes will be assessed.

B. RNA sequencing.

a. Isolation of total mRNA

b. Construction of cDNA library

c. Sequencing and bioinformatics analysis

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